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LETTER TO EDITOR

Interactions between earthworm neuroendocrine and immune systems

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To the Editor

We read with interest the recent paper about the importance of studying invertebrate immune-neuroendocrine functions published in the "Vision and Perspectives" section of Inv. Surv. J. (Ottaviani, 2014). The paper reinforces the profoundly important view that the neuroendocrine and innate defense systems of invertebrates and vertebrates can interact in concerted harmony (Cohen and Kinney, 2007). To consolidate the insights propounded in these seminal articles we would like to advocate earthworms as tractable and powerful models for studies on immune-neuroendocrine interactions. Earthworms are relatively easy to culture and manipulate in the laboratory, and deep knowledge of particular aspects of their physiology and molecular genetics is burgeoning rapidly (Stürzenbaum *et al.*, 2009; Liebeke *et al.*, 2014).

Earthworms, as representatives of oligochaete annelids, are metamerically-segmented celomates with a closed circulation and a well-developed nervous system. A segmented celomic cavity communicates with the external environment via dorsal pores, thus the celom ubiquitously contains bacteria, protozoans and fungi, as well as abundant free-floating immunocytes and humoral factors that inhibit microorganism outgrowth (Bilej *et al.*, 2011). In common with other invertebrates, earthworms are devoid of adaptive immunity based on T and B lymphocytes and antibodies; these components are present in jawed vertebrates only. However, the earthworm immune system detects the conserved Pathogen-Associated Molecular Patterns (PAMPs) of microbes by Pathogen Recognition Receptors (PRR), among them the extensively studied Toll-like receptors (Coscia *et al.*, 2011; Skanta *et al.*, 2013). Evidently, earthworms have evolved efficient innate immunity with both cellular and humoral components (Bilej *et al.*, 2011). The immunocytes, according to the term coined out by Ottaviani (2011) for invertebrate cells endowed with attributes of vertebrate macrophages, are represented in earthworms by free-floating amebocytes derived from the lining of the celomic cavity (Parry, 1975). In some lumbricid species amebocytes are accompanied by eleocytes, the latter being detached chloragocytes invested with granules containing species-specific amounts of riboflavin (vitamin B2). Riboflavin is now recognized as a potentiator of immunocompetence and tissue regeneration capacity in earthworms and other organisms (Plytycz and Morgan, 2011; Johnson *et al.*, 2012).

Earthworms stressed by predators or physical/chemical irritants expel celomocyte-containing celomic fluid through the dorsal pores during spasmic body movements. This ability is commonly exploited for non-invasive retrieval of celomocytes for *ex vivo* studies and/or for temporal depletion of earthworm celomocytes and celomocyte-derived humoral factors. Depletion of celomocytes is followed by their restoration (Eyambe *et al.*, 1973); amebocyte restoration is faster than eleocyte restoration (Klimek *et al.*, 2011; Santocki *et al.*, 2015).

The earthworm central nervous system (CNS) is a highly differentiated neuroendocrine structure which produces hormones, neurohormones and neurotransmitters (*e.g.*, Takahama *et al.*, 1998; Hartenstein, 2006; Wilhelm *et al.*, 2006; Herbert *et al.*, 2009; Molnar *et al.*, 2015a). The CNS of earthworms is comprised of a ventral nerve cord (VNC) consisting of segmentally repeated ganglia joined longitudinally by connectives and radially by comissures. The first VNC ganglia are fused to form the suboesophageal ganglion which is connected by paired circumpharyngeal connectives to a dorsal cerebral ganglion, often loosely referred to as the 'brain'. Its anatomical location makes the earthworm brain easy to remove surgically.

Earthworms possess the remarkable ability to regenerate the cerebral ganglion within a few weeks (Lubics *et al.*, 2002; Csoknya *et al.*, 2003; Okrzesik *et al.*, 2013; Molnar *et al.*, 2015b). Our experiments on adult earthworms (*Dendrobaena veneta*) have shown that amputation of the anterior (brain-containing) segments, or direct surgical brain removal, caused an immediate and pronounced inhibition of reproduction. Reproductive output

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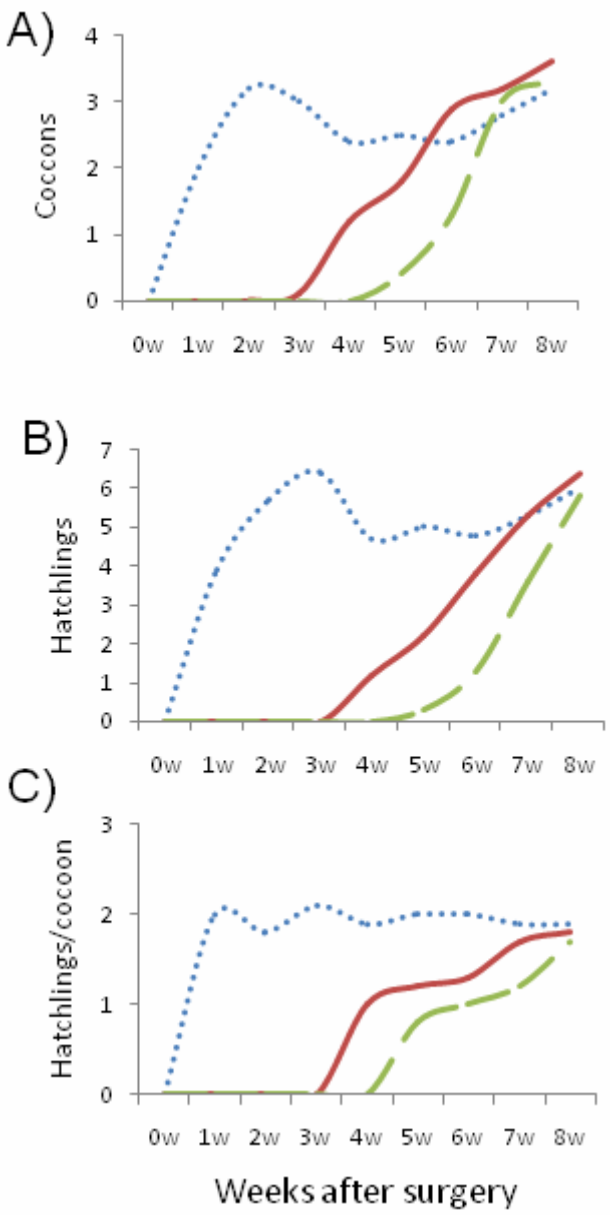

Fig. 1 Reproductive success during 8 consecutive weeks in *Dendrobaena veneta* either untreated (blue dotted lines), or after surgical brain removal (red solid lines), or surgical brain removal in celomocyte-depleted worms (green dashed lines) measured as: A) numbers of cocoons per worm; B) numbers of hatchlings per worm; C) numbers of hatchlings per cocoon. [This is a modified version of a Figure and Table published in Molnar *et al.,* 2015b]

subsequently recovered over a matter of weeks and, significantly, in tandem with restoration of brain integrity, including active neurosecretory cells. Thus, the restoration of reproductive activity is a sensitive and non-invasive biomarker for tracking the progression of brain regeneration (Okrzesik *et al.*, 2013). Our studies also included monitoring reproductive activity of unmanipulated (control) *D. veneta* and of their experimental counterparts subjected either to surgical brain extirpation only or to the dual treatment (*i.e.,* celomocyte extrusion and brain extirpation). As before, reproduction was temporarily inhibited in all brain-extirpated worms and was concomitantly restored alongside brain regeneration, both events proceeding faster in subjects with an undisturbed immune system compared with celomocyte-depleted ones (Molnar *et al.*, 2015b) (Fig. 1). On the other hand, restoration of celomocytes was slower in worms engaged in regenerating their extirpated brains then compared with counterparts possessing intact brains. Reproduction was only slightly inhibited by celomocyte depletion in otherwise intact worms (Okrzesik *et al.*, 2013; Molnar *et al.*, 2015b).

Collectively, our studies provide empirical support for the notion that the neural and immune systems of earthworms can be demonstrated to be functionally intertwined.

The earthworm experimental model that we have briefly outlined above is unquestionably amenable to detailed examination by a variety of molecular-genetic platforms and by various spatially defining immune-localization microscopic methodologies.

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References

Bilej M, Prochazkova P, Silverowa M, Jaskova R. Earthworm immunity. Adv. Exp. Med. Biol. 708: 66-79, 2011.

Cohen N, Kinney KS. Exploing the neural-immune system interactions. An update. In: Ader R (ed.), Psychoneuroimmunology, vol. 1, 4th edition, Elsevier Academic Press, Burlington MA 01803, USA, pp 1-38, 2007.

Coscia MR, Giacomelli S, Oreste U. Toll-like receptors: an overview from invertebrates to vertebrates. Inv. Surv. J. 8: 210-226, 2011.

Csoknya M, Barna J, Hiripi L, Hámori J, Elekes K. Reorganization of monoaminergic systems in the earthworm, *Eisenia fetida*, following brain extirpation. J. Exp. Zool. Part A: Comp. Exp. Biol. 296A: 18-29, 2003.

Eyambe GS, Goven AJ, Fitzpatrick LC, Venables BJ, Cooper EL. A non-invasive technique for sequential collection of earthworm (*Lumbricus terrestric*) leukocytes during subchronic immunotoxicity studies. Lab. Anim. 25: 61-67, 1991.

Hartenstein V. The neuroendocrine system of invertebrates: a developmental and evolutionary perspective. J. Endocrinol. 190: 555-570, 2006.

Herbert Z, Pollák E, Zongman A, Boros A, Kaplan N, Molnar L. Identification of novel neuropeptides in the ventral nerve cord ganglia and their targets in an annelid worm, *Eisenia fetida*. J. Comp. Neurol. 514: 415-432, 2009.

Johnson SJR, Raja SE, Vedha YB, Amutha AEAJK, Dinesh S, Durairaj SJ, *et al*. Autofluorescence in BrdU-positive cells and augmentation of regeneration kinetics by riboflavin. Stem Cells Develop. 21: 2071-2083, 2012.

Klimek M, Kruk J, Plytycz B. Restoration of coelomocytes in the earthworm *Dendrobaena veneta.* Acta. Biol. Cracov. Ser. Zool. 54: 11-17. 2012.

Liebeke M, Bruford MW, Donnelly RK, Ebbels TMD, Hao J, Kille P, *et al.* Identifying biochemical phenotypic differences between cryptic species. Biol. Lett. 2014, doi: 10.1098/rsbl.2014.0615.

Lubics A, Reglodi D, Szelier M, Lengvari I. Time-course of the regeneration of the earthworm cerebral ganglion with special reference to serotonergic elements. Eur. J. Anat. 6: 147-152, 2002.

Molnar L, Pollak E, Somogyi I, Engelmann P. On the existence of possible pituitary adenylate cyclase-activating polypeptide adenylate type 1 receptor in earthworms. Inv. Surv. J. 12: 173-175, 2015a.

Molnar L, Pollak E, Skopek Z, Gutt E, Kruk J, Morgan AJ, Plytycz B. Immune system participates in brain regeneration and restoration of reproduction in the earthworm *Dendrobaena veneta*. Dev. Comp. Immunol. 52: 269-279, 2015b.

Okrzesik J, Kachamakova-Trojanowska N, Jozkowicz A, Morgan AJ, Plytycz B. Reversible inhibition of reproduction during regeneration of cerebral ganglia and celomocytes in the earthworm *Dendrobaena veneta*. Inv. Surv. J. 10: 151-161, 2013.

Ottaviani E. Immunocyte: the invertebrate counterpart of the vertebrate macrophage. Inv. Surv. J. 8: 1-4, 2011.

Ottaviani E. The importance of studying invertebrate immune-neuroendocrine functions. Inv. Surv. J. 11: 1-3, 2014.

Parry MJ. Evidence of mitotic division of coelomocytes in the normal, wounded and grafted earthworm *Eisenia foetida*. Experientia 117: 449-451, 1975.

Plytycz B, Morgan AJ. Riboflavin storage in earthworm chloragocytes/eleocytes in an eco-immunology perspective. Inv. Surv. J. 8: 199-209, 2011.

Santocki M, Falniowski A, Plytycz B. Restoration of experimentally depleted coelomocytes in juvenile and adult composting earthworms *Eisenia andrei, E. fetida* and *Dendrobaena veneta.* Appl. Soil Ecol. 2015 [in press].

Skanta F. Roubalova R, Dvorak J, Prochazkova P, Bilej M. Molecular cloning and expression of TLR In the *Eisenia adrei* earthworm. De. Com. Immunol. 41: 694-702, 2013.

Stürzenbaum SR, Andre J, Kille P, Morgan AJ. Darwin and his earthworms: genomics, proteomics and metabolomics. Proc. R. Soc. B 276: 789-797, 2009.

Takahama H, Haibara K, Oumi T, Ukena K, Morishita F, Furukawa Y, *et al.* Immunohistochemical localizationof annetocin, an earthworm oxytocin-related peptide, and identification and ultrastructural characteristics of the annetocin-secretory cells in the oligochaete earthworm *Eisenia foetida*. Zool. Sci. 15: 381-388, 1998.

Wilhelm M, Koza A, Engelmann P, Nemeth P, Csoknya M. Evidence for the presence of thyroid-stimulating hormone, thyroglobulin and their receptors in *Eisenia fetida*: a multilevel hormonal interface between the nervous system and the peripheral tissues. Cell Tissue Res. 324: 535-436, 2006.